

CLAIMS

1. A method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:
  - a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
  - b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,
  - c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
  - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
  - e) selecting progeny lines.
2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
3. A method according to claim 1 wherein the double low cms line of spring Brassica napus of step a) is R211.
4. A method according to claim 1 wherein the testing in step d) is performed with the combination of five markers selected from PGIo1, PGIUNT, PGIint, BolJon and CP418.
5. Double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a

good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

- 5 6. Double low restorer lines of Brassica napus according to claim 5, wherein they present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
7. Brassica napus hybrid plants and progeny thereof obtained through the steps of:
  - 10 a) providing a restorer line produced according to claim 1 and bred to be homozygous,
  - b) using said restorer line in a hybrid production field as the pollinator,
  - c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
  - 15 d) harvesting the hybrid seed from the male sterile plant.
8. The seeds of Brassica plant developed from the Brassica line obtained in claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.
10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4 , 2003, under the reference number NCIMB41183.
- 25 11. Use of the combination of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.
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12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.
- 5 13. Use according to claim 12, wherein:
- The marker PGIol is amplified using the primers: PGIol U and PGIol L  
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';  
PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
  - The marker PGIint is amplified using the primers: PGIint U and PGIint L  
10 (PGIint U: 5'CAGCACTAATCTTGCGGTATG3';  
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
  - The marker PGIUNT is amplified using the primers: PGIol U and PGIint L:  
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';  
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
  - The marker BolJon is amplified using the primers: BolJon U and BolJon L:  
15 (BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';  
BolJon L: 5'GCCTACTCCTCAAATCACTCT3')
  - The marker CP418 is amplified using the primers: SG129 U and pCP418 L:  
(SG129 U: cf Giancola et al (5)  
20 pCP418 L: 5'AATTTCTCCATCACAAGGACC3' )

14. PGIol marker whose sequence follows:

25 TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60  
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120  
AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGAATCTTTC TTCATTGTTT 180  
GTTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTTC TACCGGGTCT 240  
GATGTACA 248

15. PGIUNT marker whose sequence follows:

30 TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60  
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120  
AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGAATCTTTC ACCGGGTCTG 180  
GTTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTTC TACCGGGTCT 240  
ATGTACAATG CTAGTCTCCA TGTTCTTGGG GATCATGATT TATTTTCTAC ATGTATTTCAG 300  
35 ACAGTACAGA AGAAAGTGTT CAAAACTCTG GATGTTTTAA TTTACAGTTA GTGGAGAAGT 360  
TCGGCATTGA TCCGAACAAT GCATTTGCAT TTTGGGACTG GGTGGTGGA AGGTACAGTG 420  
GTAAGTGCTT GTTTATTTGG TTGTATAAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480  
AACTGAAAT CTTTTGCAGT TTGCAGTGCT GTTGGAGTCT TACCATTGTC TCTACAGTAT 540  
GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAAATGT 600

	CTTAGGCATA	TTCTTTCTAT	TTTATTTCCC	TCTTAATGAT	TTCTTCTTTT	TTTTATTGCA	660
	TTCCCGTTTT	ATTTTCAAAA	GTTGTTACTG	TCTCTAAATC	AAGAAGAAAC	CTTCTTAGTA	720
	GATCCAGCTG	ATATTACAGC	TTTTTTAAAT	TGGACTGCAG	GTTTTTAAAG	GGGAGCTTCA	780
	AGCATTGATA	AGCATTTCCT	GTCCACACCG	TTTGAGAAGA	ATATACCCGT	GAGTTGCATT	840
5	AGTTGTGTGA	TTATACAGTT	TTCTTGTCTT	TTTGCTATGT	CCATCAACAC	TAGAGATTCTG	900
	TGAAGTTATT	AGTGTAGTCA	ACGCATAGGG	AGAGGTGATT	GGTGACTTTT	GGACGATTTC	960
	AGGTGCTTTA	GGGTTATTG					979

16. PGLint marker whose sequence follows:

10	CAGCACTAAT	CTTGCGGTAT	GAATTTGTGA	TAAATTTTGT	TTGTTTGTGA	CTCTTTCTTC	60
	ATTGTTTCGTT	TTCTGTACAAT	AAACCGAATG	TATAATCTTT	TACAACTGA	ATTTTCTACC	120
	GGGTCTGATG	TACAATGCTA	GTCTCCATGT	TCTTGGGGAT	CATGATTTAT	TTTCTACATG	180
	TATTCAGACA	GTACAGAAGA	AAGTGTTCAT	AACTCTGGAT	GTTTTAATTT	ACAGTTAGTG	240
	GAGAAGTTCTG	GCATTGATCC	GAACAATGCA	TTTGCAATTTT	GGGACTGGGT	TGGTGGGAAGG	300
15	TACAGTGGTA	AGTGCTTGT	TATTTGGTTG	TATAAATTTT	TCGTCCATTT	CCGCTTGCTT	360
	AGTGTATAAC	TGAAATCTT	TTGCAGTTT	CAGTGTCTT	GGAGTCTTAC	CATTGTCTCT	420
	ACAGTATGGC	TTCTCTGTGG	TTGAGAAGTA	CGGTACCTTC	TACTTTATCA	GCCATCTCAT	480
	AAAATGTCTT	AGGCATATTC	TTTCTATTTT	ATTTCCCTCT	TAATGATTTT	TTCTTTTTTT	540
	TATTGCATTC	CCGTTTTATT	TTCAAAAGTT	GTTACTGTCT	CTAAATCAAG	AAGAAACCTT	600
20	CTTAGTAGAT	CCAGCTGATA	TTCAAGCCTT	TTTAAATTGG	ACTGCAGGTT	TTTAAAGGGG	660
	AGCTTCAAGC	ATTGATAAGC	ATTTCCAGTC	CACACCGTTT	GAGAAGAATA	TACCCGTGAG	720
	TTGCATTAGT	TGTGTGATTA	TACAGTTTTC	TTGTCTTTTT	GCTATGTCCA	TCAACACTAG	780
	AGATTCGTGA	AGTTATTAGT	GTAGTCAACG	CATAGGGAGA	GGTGATTGGT	GACTTTTGA	840
	CGATTTTCAGG	TGCTTTAGGG	TTATTG				866
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17. BolJon marker whose sequence follows:

	GATCCGATTC	TTCTCCTGTT	GAGATCAGCT	CCAAACATCA	AACAACCTGT	ACACAAATAT	60
	CTTTACTTGC	TAAATGGAAC	ATGACAAGAG	ATAGAAAATC	TTGCTCATAG	TATTGTACAA	120
	GGGATAACAG	TGTAGAAAAC	AAACCGTCTG	TAAGATTTTC	TCCCTGATCC	TCTCACTTAA	180
30	CCAGTAGGCG	TTTTTCACAT	TGAAGCGCAT	ATCTACTTTG	GTATTCACTG	AATAAAAAAA	240
	GAAAGCTGGT	AACATGTGAA	GGATATACAA	GCATTGATAC	ACCAAGTAGT	CACAACTAC	300
	ATTATAAAGG	TCAGACCTTT	GTTACATTC	TGGCCTCCAG	GACCACCGCT	TCTAGCAAG	360
	TTAAGCGTAA	CATGGTCTGC	ACGTATACAA	ATGAAAATGT	TTCTATCAAA	ATCCTATAAA	420
	ATAGAGCTCT	ATAACATTGT	CGATACATAG	TTTCACTAAC	TCTGCAAGTA	CTAAACACAT	480
35	ATACAAACAA	AACTATGCGA	ACAGATCAAA	ACTACTACAG	AACACAGTTC	TATGACACTG	540
	TCGATAGTAA	CATCCTCTGC	AAGTACCAAA	GAGATAGCAA	ATGAAACTAT	GTAAACAAAT	600
	CAAAATTCTA	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	660
	GTAAATATTT	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	720
	TCCAACAAAA	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	780
40	ACCACTACAG	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	840
	CTTTCCCCGT	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTACGA	900
	CATCGTTTTG	AGATTAGAAC	AAACTGAAAC	TTACGTAGAG	TGATTTGAGG	AGTAGGC	957

18. CP418 marker whose sequence follows:

45	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	GTAAATATTT	60
	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	TCCAACAAAA	120
	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	ACCACTACAG	180
	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	CTTTCCCCGT	240
	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTAACG	ACATCGTTTT	300
50	GAGATTAGAA	CAAACTGAAA	CTTACGTAGA	GTGATTTGAG	GAGTAGGCTC	GTTGCCAGCA	360
	GAGCTAGCTC	TCTCCTCCGC	CTCATGAAGC	ATCTGTTGCA	CCTGAGACAA	CCGTGACGAA	420
	ACTTTCCGAT	CACCGCCACC	AGAATTCTGC	GCCGCGCATC	GGAAGGATCC	GAATCGGGAA	480
	CTGAGTGAAC	CCGAGCGATC	CCGGGAGTGC	GCCGAGCGA	TGGGAAAAGA	GAGTGGCACG	540
	ATTTGACGCA	AGAGTGGAAG	AGGAGAGGGT	GGTGGATAAA	CTCGCGTATG	ATCAAGTTCTG	600
55	TCATCGTCCT	GATTGCCGCC	ATTTTTTTTT	TCAGGGCGCT	CTGTGGCTTA	GAAGTTTCCG	660
	atgtcaatga	ac					672